HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Parasitological research doi:10.1016/S2222-1808(15)61013-1

The correlation between platelet count and parasite density in children with malaria infection

Suryani Margono, Bidasari Lubis, Syahril Pasaribu, Hendri Wijaya, Ayodhia Pitaloka Pasaribu*

Department of Pediatrics, Medical Faculty, University of Sumatra Utara, Medan, Indonesia

ARTICLE INFO

Article history:
Received 14 Sep 2015
Received in revised form 13 Jan 2016
Accepted 22 Feb 2016
Available online 14 Mar 2016

Keywords:
Malaria
Children
Platelet count
Parasitemia
Splenomegaly

ABSTRACT

Objective: To assess the correlation between platelet count and parasite density.
Methods: A cross-sectional study of febrile children was conducted in Batubara Regency, North Sumatra, Indonesia. We used rapid diagnostic tests and blood smears to identify children with malaria infection. Platelet counts and measurement of parasite density were performed manually using a microscope.
Results: A total of 72 children with positive malaria infection were enrolled in the study. Sixty one children had *Plasmodium vivax*, two children had *Plasmodium falciparum*, and nine children had mixed infection. The mean platelet count from all patients was (230 940.00 ± 96 611.53)/μL and the mean parasite density was (2 254.83 ± 1 432.55)/μL. There was a weak negative correlation between the platelet count and the parasitemia count (r = –0.145). The platelet count in the mixed infection group was significantly lower [(175 444.44 ± 48 093.95)/μL] than that in the vivax malaria group [(231 927.87 ± 99 475.28)/μL] (P = 0.024). Children with splenomegaly had a significantly lower platelet count [(189 655.56 ± 80 115.76)/μL versus (245 230.77 ± 98 380.74)/μL, P = 0.04] and a higher parasite density [(3 635.78 ± 1 623.70)/μL versus (1 789.88 ± 927.29)/μL, P < 0.000 1] than those without splenomegaly.
Conclusions: There was a weak negative correlation between the platelet count and parasite count.

1. Introduction

Malaria remains a leading cause of infection, with significant mortality and morbidity rates. It is estimated that approximately 250 million malaria cases and more than 500 000 deaths occur annually, 86% of which are children under five years of age[1]. The burden of malaria in Southeast Asia has been under appreciated, despite recent evidence suggests that the continent has approximately 40% of malaria cases worldwide[2].

Thrombocytopenia is a common sign found in patients with *Plasmodium falciparum* (*P. falciparum*) infection, but it has been recently reported in *Plasmodium vivax* (*P. vivax*) or mixed infection cases in several endemic countries[3-5]. A systematic review study reported that thrombocytopenia ranged from 24% to 94% in patients with acute malaria, and that it was considered to be one marker of malaria infection in acute febrile patients in endemic areas; in addition, it was correlated with a higher degree of parasitemia levels[6].

There has been no data on platelet count and parasite density in children with malaria in endemic region in Indonesia. Therefore, the aims of this study were to investigate the correlation between platelet count and parasite density and to determine the effect of thrombocytopenia severity on diagnosis and prognosis of malaria patients.

2. Materials and methods

2.1. Study design

An observational analytical cross-sectional design study was conducted on May to June 2015 in Lima Puluh Sub-district, Batubara Regency, North Sumatra Province, Indonesia.

2.2. Inclusion and exclusion criteria

The inclusion criteria were children 0–18 years old with fever or history of fever in the last 48 h and rapid diagnostic tests malaria

*Corresponding author: Dr. Ayodhia Pitaloka Pasaribu, Department of Pediatrics, Medical Faculty, University of Sumatra Utara , Dr. Mansyur No. 5 Street, Medan 20156, Indonesia.
Tel: +62812 602 4392
E-mail: ayodhia_pitaloka@yahoo.com

The study protocol was performed according to the Helsinki declaration and approved by the Ethics Committee of the Faculty of Medicine University of Sumatra Utara. Informed written consent was obtained from the patients' guardians.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

positive for malaria falciparum and or Pan (other malaria species such as *P. vivax*, *Plasmodium ovale*, or *Plasmodium malariae*). Rapid diagnostic tests were examined using Parascreen™ devices produced by Zephyr Biomedicals, India. We excluded children with a history of bleeding disorders, severe illnesses (*e.g.*, chronic liver disease, malignancies, and heart disease) and a history of drugs uses (*e.g.*, aspirin, chloramphenicol and chemotherapy drugs) for the previous 2 weeks prior to the data collection. Patients who fulfilled the inclusion criteria were enrolled in this study.

2.3. Data collection

Interviews were performed and questionnaires were completed by the patients' guardians; physical examinations including weight and height measurement, and spleen palpation using the Hackett methods were performed. Thick and thin peripheral blood films were obtained, stained with Giemsa and examined microscopically under oil immersion. Parasite species and parasite density were confirmed and counted by a parasitologist. Platelet counts were performed by a hematology analyst. Antimalarial therapy was administered to positive malaria patients, including dihydroartemisinin 2–4 mg/kg body weight and piperaquine 16–32 mg/kg body weight, each administered once daily for 3 days.

2.4. Ethics

The study protocol was performed according to the Helsinki declaration and approved by the Ethics Committee of the Faculty of Medicine University of Sumatra Utara. Informed written consent was obtained from the patients' guardians.

2.5. Statistical analysis

The data were presented as the means, percentages and standard deviations. Spearman correlation analysis was used to determine correlations between the platelet count and parasite density. The Mann-Whitney *U*-test was performed to assess the presence of splenomegaly and its association with both platelet count and parasite density. This study used a 95% confidence interval (*CI*), and a P value < 0.05 to denote significance. All analyses were conducted with SPSS software (version 17.0).

3. Results

A total of 320 children were screened and 72 children with positive malaria infection and who fulfilled the inclusion criteria were enrolled. Sixty-one children had vivax (85%), two children had falciparum (3%), and nine children had mixed infection (12%). The characteristics of the subjects are shown in Table 1. In a study of 72 patients were positive for malaria infection, nine (12.5%) had thrombocytopenia, five (55.56%) had vivax malaria, and four (44.44%) had mixed malaria cases.

Table 1
Characteristics of the subjects. *n* (%).

Characteristics		Subjects No. (n = 72)
Sex	Male	38 (52.8)
	Female	34 (47.2)
	Age (year)	11.10 ± 4.80
	Weight (kg)	31.01 ± 15.42
	Height (cm)	131.74 ± 21.68
Nutritional status	Mild malnutrition	14 (19.4)
	Moderate malnutrition	5 (7.0)
	Normal	47 (65.3)
	Overweight	6 (8.3)
Fever	Yes	0 (0)
	No	72 (100.0)
Anemic	Yes	59 (81.9)
	No	11 (15.3)
Splenomegaly	Yes	19 (26.4)
	No	53 (73.6)

Using Spearman's correlation analysis, the results showed that there is a weak negative correlation between the platelet count and parasite density among all study subjects (r = –0.145) and in the group of patients with malaria vivax infection (r = –0.225). However, in the group of patients with mixed infection, we found a strong positive correlation (r = 0.844) between the platelet count and parasite density (P = 0.004, Table 2, Figures 1–3).

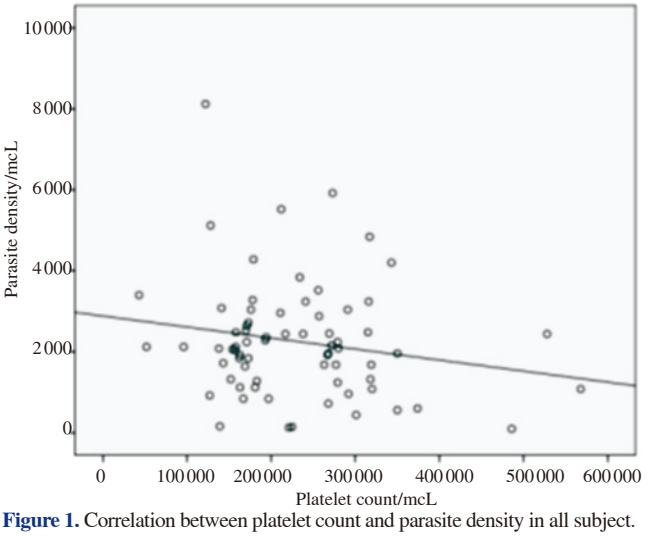

Figure 1. Correlation between platelet count and parasite density in all subject.

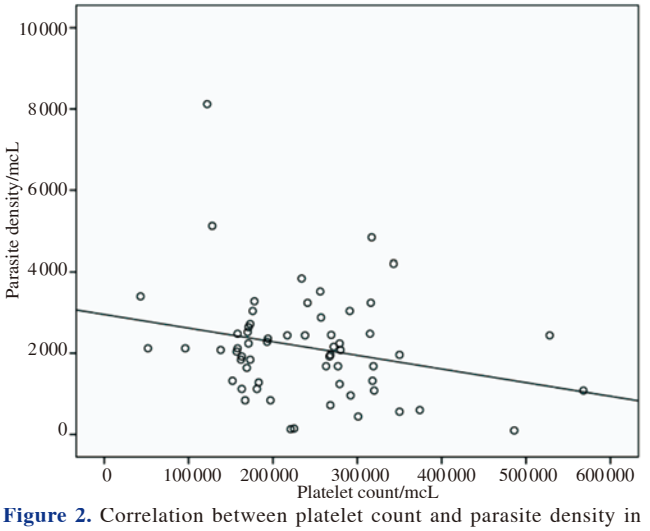

Figure 2. Correlation between platelet count and parasite density in malaria vivax infection group.

Table 2
Correlation between platelet count and parasite density.

Patients	No.	Platelet count/μL	Parasite density/μL	*P*	*r* (correlation)
All subject	72	230 940.00 ± 96 611.53	2 254.83 ± 1 432.55	0.230[a]	–0.145
P. vivax infection	61	231 927.87 ± 99 475.28	2 151.11 ± 1 323.36	0.081[a]	–0.225
Mixed infection	9	175 444.44 ± 48 093.95	2 957.78 ± 1 981.83	0.004[b]	–0.844

[a]: Spearman; [b]: Pearson.

The platelet count in subjects with mixed infection malaria was lower [(175 444.44 ± 48 093.95)/μL] compared to the patients with vivax infection [(231 927.87 ± 99 475.28)/μL]. The results of the Mann-Whitney *U*-test showed that there was a significant difference in the mean platelet count based on type of malaria infection (Figure 4).

Using the Hackett examination method, the mean platelet count in the group of subjects with splenomegaly was (189 655.56 ± 80 115.76)/μL, which was lower than in the group of subjects without splenomegaly [(245 230.77 ± 98 380.74)/μL]. The Mann-Whitney *U*-test results showed that there was a significant difference in the platelet count between the patients with and without splenomegaly (*P* = 0.04, Figure 5). However, we did not find any difference in the platelet count using Hackett I and II [(190 215.38 ± 73 773.10)/μL versus 199 500.00 ± 97 592.520/μL, *P* = 0.629].

With the Hackett method, there was a significant difference in parasite density based on the presence of splenomegaly. The mean parasite density was higher in the group of malaria patients with splenomegaly [(3 635.78 ± 1 623.70)/μL] compared with those without splenomegaly [(1 789.88 ± 927.29)/μL], using the Mann-Whitney *U*-test (*P* < 0.000 1, Figure 6).

There were differences in parasite density using the Hackett

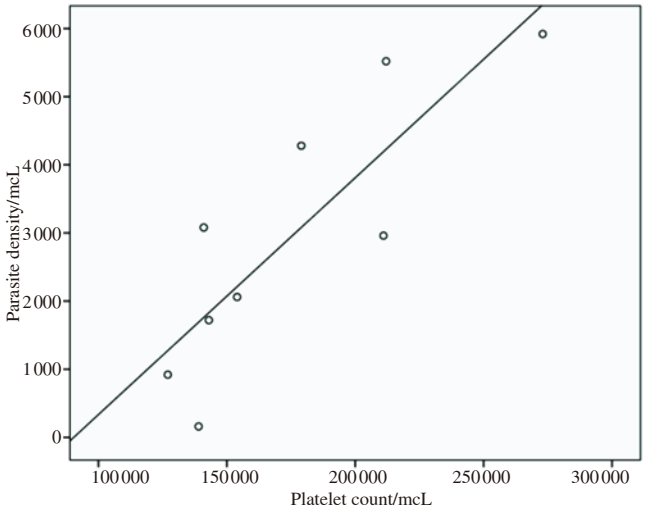

Figure 3. Correlation between platelet count and parasite density in the mixed infection group.

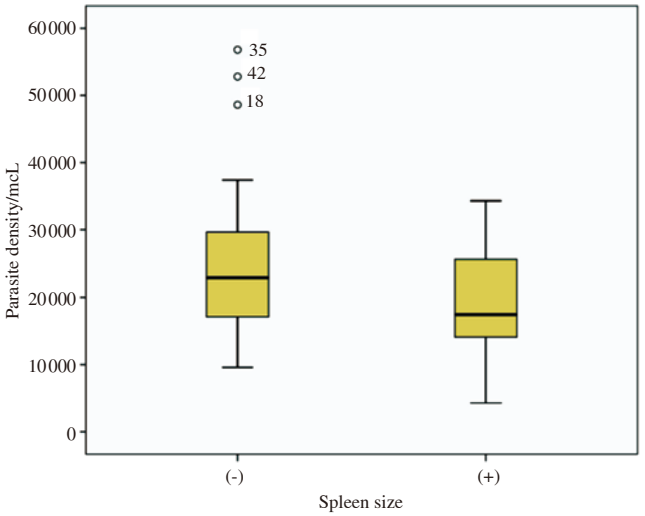

Figure 5. Difference in platelet count based on splenomegaly with the Hackett method.

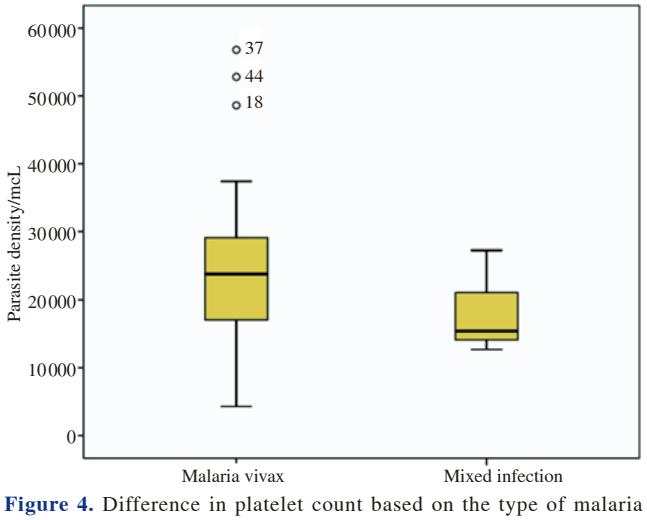

Figure 4. Difference in platelet count based on the type of malaria infection.

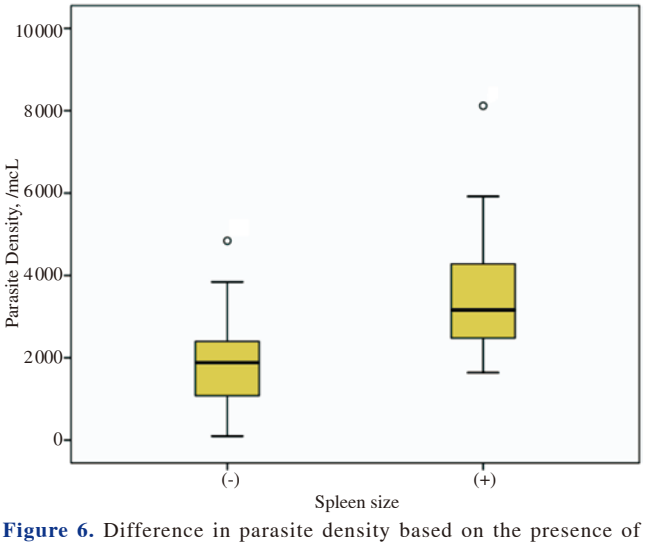

Figure 6. Difference in parasite density based on the presence of splenomegaly using Hackett method.

method. The mean parasite density was higher in the group of patients with more severe splenomegaly [Hackett I (3 089.50 ± 1 097.24)/μL versus Hackett II (4 920 ± 1 970/μL, P = 0.029]. A weak positive correlation was observed between the platelet count and the patient's age using Spearman's correlation analysis (r = 0.125, P = 0.304, Figure 7).

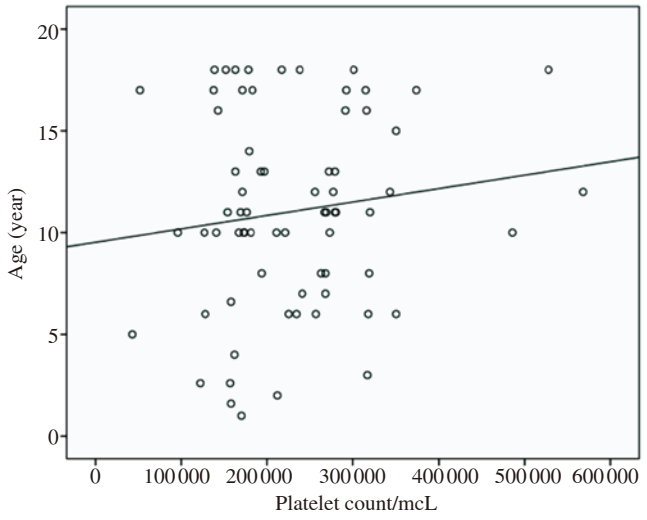

Figure 7. Correlation between platelet count and patient's age.

4. Discussion

Malaria infection remains a major public health problem in tropical countries worldwide[1], including the Batubara District, Indonesia. *P. falciparum*, *P. vivax*, and mixed infection of both types of malaria infection are found in Batubara District. In our study, prevalence of *P. vivax* was 85%, followed by mixed infection at 12% and *P. falciparum* 3%. In this study, males numbered 52.8%, and females were 47.2%. The prevalence of male might have been because of their nocturnal outdoor activities[7].

Thrombocytopenia is a common sign in malaria infection, which occurs in 24%–94% of malaria cases[6]. The pathogenesis of malaria thrombocytopenia is complex and it may be related to coagulation disturbances, splenomegaly and platelet destruction by macrophages, bone marrow alterations, antibody-mediated platelet destruction, oxidative stress and platelet aggregation[6]. Although thrombocytopenia is common, hemorrhagic events are rare and are usually associated with severe thrombocytopenia or disseminated intravascular coagulation[8,9].

A study conducted in Papua, Indonesia, revealed that more than 50% of patients with high parasite density of more than 10% had platelet count below 50 000/μL[10]. In this study, we found only nine patients (12.5%) with thrombocytopenia (from total 72 patients), which is lower than previous studies[10]. This finding might be caused by a relatively low parasite density in the blood. The overall parasite density of the subjects in our study was only approximately 0.2%. Because of the relatively low parasite density in this study, the platelet count did not fall below 150 000/μL.

A study in Kenya found a strong negative correlation between platelet count and parasite density in patients with malaria falciparum infection[11]. In contrast to that finding, a study in Brazil found that patients with malaria vivax infection had a weak negative correlation[12]. Similar to that study, we also found a weak negative correlation between platelet count and parasite density. We estimate that this result was because most of the samples were malaria vivax. Previous research in Papua, Indonesia, has also found a weak negative correlation in the malaria vivax group[10]. Consistent with a study in Kenya[11], the Taylor *et al.* also found a strong negative correlation between platelet count and parasite density in the malaria falciparum group[10].

Interestingly, we observed a different finding in the mixed infection group, that is, the platelet count and parasite density showed a strong positive correlation. This finding could be due to the relatively small sample size, therefore the result might not be representative. Another reason is likely because some patients may have consumed antimalarial drugs, which might have changed the platelet count and parasite density in the blood.

In this study, the platelet count in the mixed infection group was significantly lower than that in monoinfection group. This result aligned with the theory that *P. falciparum* attack erythrocyte of all ages, while *P. vivax* usually only attack young erythrocytes[13]. This finding may explain why the mixed malaria infection had a higher parasite density, thereby resulting in more frequent and lower thrombocytopenia[14]. In accordance with this theory, a study in India has revealed that severe thrombocytopenia is more common in malaria falciparum than in vivax[15]. Our study cannot compare the severity of thrombocytopenia between the malaria falciparum and vivax group because of the small sample size of the falciparum group.

Hematopoiesis is one function of the spleen. The changes in the structure of the spleen during malaria infection can disrupted hematopoiesis, which will decrease the platelet count in the blood due to decreased production[16]. In this study, malaria patients with splenomegaly had a significantly lower platelet count. This finding is consistent with a Pakistan study of adult patients with malaria vivax infection[17].

The spleen captures erythrocytes that have been damaged or infected from circulation. Infected erythrocytes will be broken down and undergo spleen phagocytic clearance, causing edema in pulp and spleen lymphoma[18], due to the spleen's function in controlling malaria infection[16]. Consequently,

spleen enlargement will worsen with higher parasitemia. To confirm this theory, we found an association between parasite density and splenomegaly in this study. Our subjects with higher parasite density had more severe splenomegaly. A study in Mandailing Natal, North Sumatra, Indonesia, also found a significant positive correlation between the parasite density and the degree of splenomegaly (using the Hackett method) in malaria falciparum patients[19].

This study has several limitations. First, the malaria falciparum group had a small sample size; therefore, the results could not be analysed. Second, we examined the platelet counts manually, which could cause inaccurate calculations. A prospective cohort study design with a larger sample size is required to find the correlation between platelet count and parasite density, based on the species of malaria, and to observe the fluctuations in platelet counts after antimalarial treatment.

There was a weak negative correlation between platelet count and parasite count.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are grateful to the midwifes of Lima Puluh District for their enthusiastic participation in this study. The support provided by the principals and parents of these children was also invaluable.

References

[1] World Health Organization. World malaria report 2011. Geneva: World Health Organization; 2011, p. 66-75. [Online] Available from: http://www.who.int/malaria/world_malaria_report_2011/9789241564403_eng.pdf [Accessed on 10th January, 2016]

[2] Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 2005; **434**: 214-7.

[3] Mitra S, Abhilash K, Arora S, Miraclin A. A prospective study from South India to compare the severity of malaria caused by *Plasmodium vivax*, *P. falciparum* and dual infection. *J Vector Borne Dis* 2015; **52**(4): 281-6.

[4] Kochar DK, Das A, Kochar A, Middha S, Acharya J, Tanwar GS, et al. Thrombocytopenia in *Plasmodium falciparum*, *Plasmodium vivax* and mixed infection malaria: a study from Binaker (Northwestern India). *Platelets* 2010; **21**(8): 623-7.

[5] Metanat M, Sharifi-Mood B. Malaria vivax and severe thrombocytopenia in Iran. *Iran J Parasitol* 2010; **5**: 69-70.

[6] Lacerda MV, Maurão MP, Coelho HC, Santos JB. Thrombocytopenia in malaria: who cares? *Mem Inst Oswaldo Cruz* 2011; **106**(Suppl 1): 52-63.

[7] Mugwagwa N, Mberikunashe J, Gombe NT, Tshimanga M, Bangure D, Mungati M. Factors associated with malaria infection in Honde Valley, Mutasa District, Zimbabwe, 2014: a case control study. *BMC Res Notes* 2015; **8**(1): 829.

[8] Chaudary SC, Sonkar SK, Kumar V, Gupta A. *Falciparum malaria* presenting as subdural hematoma. *J Assoc Physicians India* 2011; **59**: 325-6.

[9] Sailo L, Pradhan D, Nongthombam R, Bhattacharyya P. Disseminated intravascular coagulation in malaria: a case report. *Niger Med J* 2014; **55**(2): 171-2.

[10] Taylor WRJ, Widjaya H, Basri H, Ohrt C, Taufik T, Tjitra E, et al. Changes in the total leukocyte and platelet counts in Papuan and non Papuan adults from Northeast Papua infected with acute *Plasmodium vivax* or uncomplicated falciparum malaria. *Malar J* 2008; **7**: 259.

[11] Maina RN, Walsh D, Gaddy C, Hongo C, Waitumbi J, Otiento L, et al. Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya. *Malar J* 2010; **9**(Suppl 3): S4.

[12] Leal-Santos FA, Silva SBR, Crepaldi NP, Nery AF, Martin TOG, Alves-Junior ER, et al. Altered platelet indices as potential markers of severe and complicated malaria caused by *Plasmodium vivax*: a cross-sectional descriptive study. *Malar J* 2013; **12**: 462.

[13] Kimbi HK, Sumbele IU, Nweboh M, Anchang-Kimbi JK, Lum E, Nana Y, et al. Malaria and haematologic parameters of pupils at different altitudes along the slope of Mount Cameroon: a cross sectional study. *Malar J* 2013; **12**: 193.

[14] Gupta NK, Bansal SB, Jain UC, Sahare K. Study of thrombocytopenia in patients of malaria. *Trop Parasitol* 2013; **3**: 58-61.

[15] Patel P, Patel M, Gmait B, Modi J, Kevadiya S, Padsala S. Thrombocytopenia in malaria: correlation with various prevalent species. *Int J Med Sci Public Health* 2013; **2**: 946-50.

[16] Engwerda CR, Beattie L, Amante FH. The importance of spleen in malaria. *Trends Parasitol* 2005; **21**: 75-80.

[17] Arshad AR, Arshad A. Impact of splenomegaly on complete blood counts in hospitalized patients with *Plasmodium vivax* malaria. *Rawal Med J* 2013; **38**(3): 223-5.

[18] Urban BC, Hien TT, Day NP, Phu NH, Roberts R, Pongponratn E, et al. Fatal *Plasmodium falciparum* malaria causes spesific patterns of spenic architectural disorganization. *Infect Immun* 2005; **73**: 1986-94.

[19] Lailani D, Lubis M, Lubis B, Pasaribu S. Correlation between parasitemia and spleen size in patients with *P. falciparum* infection. *Paediatr Indonesia* 2004; **44**(5-6): 111-6.